Date: Thu, 07 Nov 2002 11:30:16 -0500
From: David Hansen <dehansen@amherst.edu>
To: lederberg@rockefeller.edu
Subject: [Fwd: imprints]

Dear Josh,
I've just realized that I may have originally sent this to an outdated e-mail address.

With best wishes,
David

Dear Josh,
Many thanks for your two articles, which arrived today. I've just finished reading them and am especially intrigued by your reexamination of (s)elective vs. instructional theories. While I certainly agree that DNA replication might well be viewed as selective, I'm not so sure about

enzymatic catalysis. Although I cannot put my finger on the key point at the moment (which most surely is your point!), my intuition says that enzymes do not merely simply "select" the transition states that are already present, but rather "instruct" their formation once the substrate(s) are bound. Let me ponder this a bit and I'll be back in touch.
My interest in imprints, in fact, pertains to the creation of new catalysts, and I've attached a brief abstract that outlines our approach. I've also pasted in below the specific aims from my NIH grant on our imprint work.

With best wishes,
David

P.S. Your recent piece in Immunological Reviews is beautifully crafted and serves as a quintessential example of the "liberal arts" approach to science that I strive to inculcate in our students. In this vein, I have also attached a small piece I wrote for C&E News last year that you might find amusing.

The objective of the research proposed is the generation of organic, polymeric molecular imprints with noveland improvedcatalytic activities. To create a molecular imprint, a template molecule is first dissolved in a solution of monomers. These monomers also contain chemical functionality that bonds either noncovalently (typically, via hydrogen or ionic bonds) or covalently (via reversibly cleavable bonds such as imines or boronic esters) with the template molecule. Formation of these bonds prearranges the monomers around the template. At this point, the mixture is copolymerized in the presence of a free-radical initiator and a large excess of crosslinker to yield a macroporous plastic. The template molecule is then washed away from the polymer, leaving microscopic cavities (the imprints) that are electrostatically and geometrically complementary to the template molecule. The resultant molecular imprint can selectively bind the template molecule (in preference to its enantiomer, for example) or can act as a catalyst if the template molecule is appropriately designed (if it is a transition state analogue, for example).

However, while molecular imprints with exquisite binding specificities can now be routinely

generated, those with catalytic activity have displayed only modest rate accelerations. Clearly, if catalytic imprints are to be truly useful, then far larger accelerations must be achieved. In an effort to generate these more efficient imprints, three new systems are proposed in the sections that follow. Each of the these systems incorporates two features, both intended to substantially enhance the catalytic activity of the imprints obtained: one, each imprint will be generated covalently; and two, each will incorporate a nucleophilic functionality in the catalytic mechanism. Covalent imprinting should allow for more precise positioning of the template (and thus of the analogous substrate) within the polymeric matrix. And direct participation of a catalytic nucleophilic functionalitya mechanistic feature that can lead to huge rate accelerations in intramolecular model systems and a key to the rate accelerations effected by the best antibody catalysts isolated to dateshould lead to enhanced rates. Whether this dual approach will in fact yield catalytic imprints with activities superior to those obtained previously is the hypothesis to be tested by the proposed work.

Specifically, the first of these systems involves the use of an imprint against a phenyl pyranoside template as a catalyst for the lactonization of the corresponding aldonic ester and amide; the second, the use of a Kemps triacid derivative as the template to generate an imprint catalyst for the hydrolysis of the corresponding acyclic diacid-amides; and the third, the use of a vinylogous amide template to generate a catalyst for the aldol condensation of the corresponding aldehyde/ketone pair.

CATALYTIC MOLECULAR IMPRINTS FOR INTRAMOLECULAR CYCLIZATION REACTIONS

ML Dougan, KG Poulin, JA Kaplan, K Solt, JL Chin, LE Richetti, GE Cruz–Schiavone, DS Delgado, SM Miller, and DE Hansen
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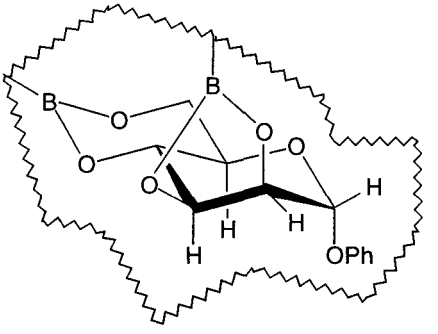
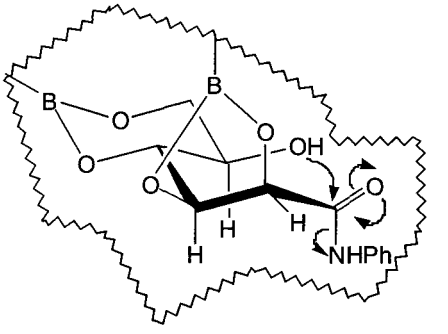
In an attempt to generate molecular imprints with improved catalytic activities, we are pursuing two novel systems. Each involves the catalysis of an intramolecular, nucleophilic cyclization reaction. The imprint is first generated against an inert, cyclic template and is then assayed for activity with the analogous acyclic substrate. Upon binding to the imprint, the acyclic substrate should adopt a reactive conformation and catalysis may ensue.

In the first of our systems, we are testing a covalent diboronate imprint against phenyl α–D–mannopyranoside as a catalyst for the lactonization of the corresponding mannonic amide (this imprint was originally generated by the Wulff group[1], which has kindly provided us with a sample):

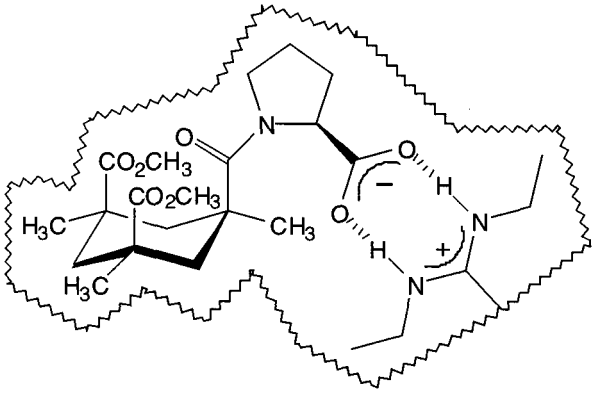


Imprint with cyclic template bound

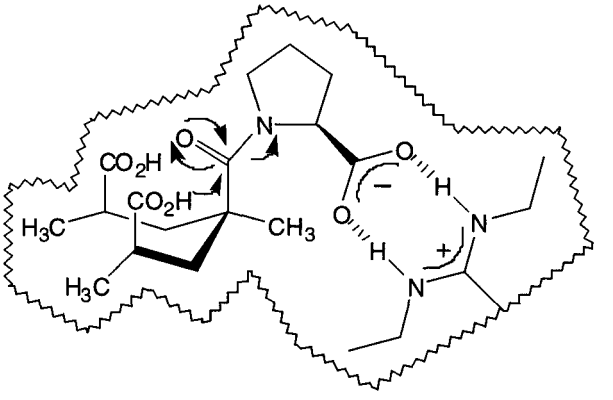


Imprint with acyclic substrate bound

The second of our systems is inspired by a Kemp's diacid–amide derivative that, as demonstrated by Menger and Ladika[2], undergoes amide cleavage 10^{10}–fold faster than a standard amide bond. We are employing an analogous, inert Kemp's *diester*–amide as a template to generate a "stoichiometric noncovalent" imprint, which we will assay for catalysis with the corresponding acyclic diacid–amide as the substrate:



Imprint with cyclic template bound



Imprint with acyclic substrate bound

1. G Wulff and S Schauhoff (1991), Racemic Resolution of Free Sugars with Macroporous Polymers Prepared by Molecular Imprinting. Selectivity Dependence on the Arrangement of Functional Groups verses Spatial Requirements, *J. Org. Chem.*, 56, 395–400.
2. FM Menger and M Ladika (1988), Fast Hydrolysis of an Aliphatic Amide at Neutral pH and Ambient Temperature. A Peptidase Model, *J. Am. Chem. Soc.*, 110, 6794–6796.

The future of chemistry? Looking forward, I can with assurance make only one prediction: the discipline, with its shifting borders and with the crosscurrents of astronomy, biology, geology, and physics swirling through it, will always intrigue me. While one might confidently surmise that nanotechnology or proteomics or climate models, to name an obvious few, will occupy our interest and yield practical benefits well into the future, a perusal of an issue of *Science* or the *Journal of the American Chemical Society* published, say, 30 years ago might suggest otherwise. The articles and communications contained therein, many describing beautiful and elegant work, are evocative of a different (although certainly not disjunctive) era. So, while I'm reluctant to speculate on precisely what chemists will be doing years from now, I am unhesitating in my belief that it will captivate. Why this conviction? Because chemistry, both in the depths of its investigations and its visions, continually surprises–in every year of my professional career, I have found delight in apprehending principles that hitherto had eluded me and in witnessing trailblazing advances. Two examples.

While preparing to teach organic chemistry a few years back, I thought I'd attempt to resolve a quandary that had been plaguing me for some time: is valence bond or is molecular orbital theory the more fundamental, the more correct, model of bonding? I reread the standard textbook explanations but then came across the following in Melvin Hanna=s *Quantum Mechanics in Chemistry*: "In more complex systems, the *states* of the atom or molecule are often expressed as products or sums of products of orbitals, but it must be kept in mind that such descriptions are convenient approximations in which a many–electron wave function is described by a product of one–electron wave functions. In the best calculation that has been done on the helium atom, all traces of atomic orbitals have disappeared." *Have disappeared*? This was a stunner. Given the successes of the *Aufbau* principle, of photoelectron spectroscopy, of frontier–orbital theory... well, of all of chemistry as I knew it, how could multi–electron species not contain orbitals? The ontological implications were staggering. With the help of my physical–chemistry colleagues, who, of course, have long been in the know, my orbital crisis did come to an end (and I was introduced to density–functional theory)–and in the process, chemistry's grip upon my imagination became only tighter.

A thrilling advance is the recent structural work published in the August 10, 2000 issue of *Nature* that, as summarized by Werner Kühlbrandt, has led to "the first complete motion picture of bacteriorhodopsin in action." This delighted not because the achievement provoked foundational tremors, but rather because it provides a quintessential illustration of the power of integrating chemistry with both biology and physics. Beginning with the isolation of bacteriorhodopsin from the purple membrane of *Halobacterium salinarum* (known then as *H. halobium*) by Walter Stoeckenius and Robert Rowan in the mid >60s, the study of this seven–helix, transmembrane protein has led to pioneering innovations in a range of techniques, from electron microscopy and x–ray crystallography to FTIR spectroscopy to site–directed mutagenesis. The full appreciation of the complex multistep, vectorial pumping mechanism has demanded the application of fundamental tenets of thermodynamics and kinetics, not to mention photochemistry. Mix in the wonder of halobacterial bioenergetics, the unexpected discovery last year that a widely distributed group of marine ?–proteobacteria employ a related light–driven rhodopsin pump, and Stoeckenius and Efraim Racker's still breathtaking use of bacteriorhodopsin in their validation of the Mitchell hypothesis, and one must stand in awe.

Chemistry enthralls, then, by the sweep of its endeavors–and by its relentless expansion into new arenas. Does this view demean the field? I think not and would quote Bernadette Bensaude–Vincent and Isabelle Stengers, who in their provocative *A History of Chemistry*, write: "Here is a body of knowledge with multiple facets and innumerable ramifications: it applies in the depths of the Earth as well as in space, and it is as important to agriculture as it is

to heavy and fine industry and to pharmacy. Here is a science that spans the borders between the inert and the living, between the microscopic and the macroscopic. How can we assign an identity to a discipline that seems to be everywhere and nowhere at once?" From this perspective, chemistry, ever protean, truly is the central science, forever embracing questions of great moment. Given its 125 years of exemplary leadership, the American Chemical Society will undoubtedly continue to play a pivotal role in fostering the explorations of chemists, explorations that, upon second thought, will surely involve nanotechnology and proteomics and climate models for many, many years to come!